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## (54) Calibration solution and calibration method for sensor.

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FR-A- 2 436 991
US-A- 3 681 255
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## Description

### Background of the Invention

This invention relates to a calibration solution and a calibration method used when measuring ion concentration in medical fields, clinical fields and biochemical fields dealing with blood and other body fluids and ion concentration in variable partial gas pressure systems using an ion sensor or gas sensor having solid electrodes.

When measuring the pH and partial gas concentration as of blood and other body fluids as solution under examination, use is usually made of a standard buffering solution prescribed in the JIS (Japanese Industrial Standard) standards and NBS standards (National Bureau Standard), e.g., phthalic acid salt, phosphate, etc., and measurement was done by using glass electrodes. In this case, electrode of the same kind and same structure as inner electrode of the glass electrode is used as reference electrode.

Solutions used for the calibration of analyses for measurement of blood gases are disclosed in FR 2 436 991.

US 3 681 255 relates to calibration fluids with predetermined dissolved gas contents for calibrating pH and gaz analyzers.

A standard solution for simultaneously calibrating a plurality of ion electrodes for determining a plurality of ions is disclosed in US 4 626 512.

It is found that ion selective electrodes as well minute solid microelectrodes and the like can be extensively utilized for medical sensors, and there are indications of practical use of pH sensors and other ion sensors in the field of clinical chemistry and artificial organ control monitors. When it is intended to apply sensors in medical field, high accuracy measurement and temperature compensation requirement levels are higher than in the case of the general analysis, and it is difficult to meet these levels.

pH sensors and other ion sensors measure the electrode potential difference corresponding to ion activity. The ion activity is the product of the ion concentration and activity coefficient. Usually, a glass membrane electrode is used as pH electrode, but can not meet the definition of pH given as

$$pH = -\log a_{H^+} \quad (1)$$

This is so because it is impossible to obtain this value strictly due to presence of liquid junction potential in contrast electrode solution section and ion activity coefficient which is incapable of measurement. For this reason, a pH value which can be mathematically calculated from pH values of the inner solution and outer measurement solution, is defined and used, which is given as

$$pH_x - pH_s = \frac{E_X - E_S}{(2.303RT/F)} \quad (2)$$

where R is the gas constant, T is the absolute temperature, F is the Farady constant, $E_x$ and $E_s$ are battery elecromotive forces in solutions X and S. The battery is composed of Pt; $H_2$ solution X or S/saturated KCl solution, saturated calomel electrode.

Here, solution with $pH_s$ is a standard solution. As the standard solution, a 0.05M potassium hydrogen phthalate solution is used, and the pH there is defined to be 3.998 (0°C, 4.000) at 15°C. This standard conforms to NBS and is adopted in Japan.

With a membrane electrode other than glass membrane, the measurement of electrode potential difference on the membrane surface is influenced by coexistent matter in the solution under examination such as other ions than the subject of measurement, protein, amino acid. This is so because the electrode potential difference is measured to calculate pH on the basis of the definition of equation (1).

In the measurement where the solution under examination is blood or like body fluids, a high accuracy of measurement is required despite slight ion concentration changes. Therefore, with calibration of sensor on the basis of the usual method of measurement as noted above, the measurement errors are too large to expect a high accuracy sensor, particularly in the medical field.

### Summary of the Invention

An object of the invention, accordingly, is to provide a calibration solution and a calibration method, which permit high accuracy measurement using sensors in medical and other fields.

To attain the above object of the invention, the invention is predicated in the fact that the activity coefficient noted above is determined by the charge of ions and total ionic strength in the solution.

The ionic strength I and activity coefficient are related to each other as a Debye-Huckel threshold equation

$$-\log(\gamma_i) = \frac{AZ_i^2\sqrt{I}}{1 + B_{a_i}\sqrt{I}} \quad (3)$$

where A and B are constants determined by the dielectric constant and temperture of the solvent, $Z_i$ an ion valency, I ionic strength and $a_i$ effective radius of hydration ion.

The activity coefficient of certain ion depends on the total ionic strength of that solution. Therefore, the ionic strength, i.e., activity coefficient, varies with changes in the concentrations of coexistent ions other than the measurement ion even if the measurement ion concentration is constant. According to the invention, the ionic strength is set to be substantially identical with the ionic strength of solution under measurement.

According to the invention, there is basically provided a calibration solution for a sensor with a solid electrode for measuring ion concentration of a solution under examination, characterized in that said solution comprises a standard buffer solution which is a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 ratio or in a 4 to 1 ratio, NaCl being added to said standard buffer solution to provide an ionic strength substantially identical with the ionic strength of the solution under examination.

The calibration solution of the above constitution permits measurement with less error. Particularly, the invention can be more suitably utilized in medical and like fields, in which the solution under examination is blood or like body fluids subject to less ion concentration changes.

According to the invention, there is also provided a calibration method for calibrating a sensor, in which at least two reference calibration solutions having different pH values are prepared, the electromotive forces of the sensor in these reference calibration solutions are measured by immersing the sensor in these solutions, and a calibration formula (calibration curve) from the electromotive forces with respect to the pH values of the reference calibration solutions.

By adopting the calibration solution noted above it is possible to obtain high accuracy measurement using a pH sensor or like sensor.

According to the invention, there is further provided a calibration solution for calibrating a sensor in case of measuring partial gas pressure as well as ion concentration of a solution under examination containing carbon dioxide gas and/or oxygen gas, said calibration solution comprising a standard buffer solution which is a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 ratio or in a 4 to 1 ratio, in which a predetermined quantity of bicarbonate buffer solution is added to the standard buffer solution to maintain a substantially constant ion concentration as well as adding NaCl to set an ionic strength substantially identical with the ionic strength of the solution under examination.

This calibration solution can be utilized for calibrating a carbon dioxide gas sensor as well as a pH sensor or like ion sensor, that is, it is possible to obtain a simultaneous calibration solution permitting high accuracy simultaneous measurement.

According to the invention, there is further provided a calibration method for calibrating an ion sensor and a gas sensor, in which at least two reference calibration solutions having different pH values and partial gas pressure values are prepared, the electromotive forces of these sensors in these reference calibration solutions are measured by immersing the sensors in the solutions, and calibration formulas are calculated by plotting the electromotive forces of the sensors for the pH values and partial gas pressure values for gas sensor.

By adopting this calibration method, measurement of ion concentration with a pH sensor or like ion sensor and measurement of partial gas pressure of carbonate or like gas with a gas sensor can be done simultaneously and with better accuracy using pertinent calibration formulas.

## Brief Description of the Drawings

Fig. 1 is a graph showing the relation between the sensor electromotive force and pH value produced according to the ionic strengths of different examples in a first embodiment of the invention;

Figs. 2 and 3 are graphs for determining $NaHCO_3$ concentrations at different pH value suited for simultaneous calibration of ion sensor and gas sensor in a second embodiment of the invention; and

Figs. 4(a) and (b) to 6(a) and (b) are views of the neighborhood of membran surface for explaining the status of charges induced on electrode membrane surface by ions and/or protein in a third embodiment of the invention together with showing of graphs.

## Detailed Description of the Preferred Embodiments

According to the invention, a first standard buffer solution is prepared by using a mixture solution composed of $Na_2HPO_4$ (27.2 mEq/l)/$NaH_2PO_4$ (6.8 mEq/l) and $Na_2HPO_4$ (22.5 mEq/l)/$NaH_2PO_4$ (22.5 mEq/l) of phosphate buffer solution system with respective pH values of 7.4 and 6.8. By adding NaCl to the standard

buffer solution, the ionic strength is changed. That is, even if the $Na^+$ ion concentration in the phosphate buffer solution is constant, the ionic strength is changed with the dissociation of the coexistent NaCl solution as given by an equation

$$pH = pK_a + \log\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} - (2n-1)\{0.5091\,(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.1I)\} \quad (4)$$

where :

$I$ (ionic strength) $= \frac{1}{2}\Sigma Z_i^2 \cdot Ci$

pKa: dissociation constant of phosphate

Zi: Ion valency

Ci: Ion mol concentration

n: an index, which is given for buffer solution as

$$H_nA^{n-} \rightleftharpoons H_{n-1}A^{(n-1)-} + H^+$$

n = 1 when

$$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$$

At least two standard buffer solutions having an ionic strength substantially identical with that of solution under examination and having different pH values are prepared on the basis of equation (4), and a calibration formula is calculated by plotting the electromotive forces of a pH sensor or the like in these standard buffer solutions by immersing the sensor in the solutions.

Thus, calibration solution and calibration method are obtained for pH sensors and like ion sensors.

Where the whole human blood is dealt with as solution under examination, the ionic strength is 0.08 to 0.18 although it varies with individuals. In this case, therefore, a calibration solution may be prepared which has an identical ionic strength.

Secondly, the preparation of calibration solution and method of calibration, which can meet an aim of simultaneously calibrating an ion sensor and a gas sensor for measuring ion concentration and partial gas pressure, respectively, will now be described.

When $CO_2$ gas, for example, is dissolved in solution, for instance, the $[H^+]$, i.e., pH, is changed such that

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

By using bicarbonate buffer solution like phosphate buffer solution, the pH of the solution can be held constant in the presence of $CO_2$.

In the bicarbonate buffer solution the carbonate dissociation constant K is given as

$$K = \frac{[HCO_3^-][H^+]}{[CO_2]}$$

From the Henry's law,

$$K = \frac{[HCO_3^-][H^+]}{\alpha P_{CO_2}}$$

where:

$\alpha$ : dissolution coefficient

$P_{CO_2}$: partial pressure of $CO_2$

By taking the logarithm of both sides,

$$pH = pK + \log\frac{[HCO_3^-]}{\alpha P_{CO_2}} \quad (5)$$

It will be seen that for the preparation of a calibration solution with a constant pH it is necessary to maintain a constant concentration of $[HCO_3^-]$ and a constant partial pressure $P_{CO_2}$.

The concentration of $[HCO_3^-]$ may be obtained from the intersection between equations (4) and (5) by varying it with the standard buffer solution system of pH values of 7.4 and 6.8 while holding constant the $[HPO_4^{2-}]/[H_2PO_4^-]$ in equation (4) and $P_{CO_2}$ in equation (5).

The invention is further predicated in charges induced on the membrane surface of a solid membrane electrode by charges on ions and protein dissolved in solution under examination.

More specifically, it is found that charges on ions and/or protein in the solution under examination have influence on the surface of the solid membrane. It is experimentarily confirmed that this phenomenon is due to the fact that NaCl electrolyte is predominant. The invention seeks to provide a calibration solution for a sensor, with which a system obtained by adding NaCl to a standard buffer solution and a system of ions and/or protein dissolved in the solution under examination are made identical in the status of charges to that of charges induced on solid membrane surface.

Now, a first embodiment of the invention, concerning calibration of a sole pH sensor, a second embodiment

concerning simultaneous calibration of a pH sensor and a gas sensor, and a third embodiment concerning calibration for making the ionic strength identical with the status of charges on ions and/or protein dissolved in the solution under examination will now be described with standard to the accompanying drawings.

First Embodiment

As shown in Tables 1-A and 1-B, calibration solution compositions with different ionic strengths of 0.05, 0.10, 0.16 and 1.0 were calculated according to equation (4) as Examples 1 to 8 in two groups, one with pH in the neighborhood of 7.4 (Table 1-A) and the other with pH in the neighborhood of 6.8 (Table 1-B).

The ratio $Na_2HPO_4/NaH_2PO_4$ in the standard buffer solution was set to 4/1 in Examples 1 to 4 and to 1/1 in Examples 5 to 8.

By plotting the relation between the electromotive force and pH value from the above tables, a calibration curve corresponding to each ionic strength as shown in Fig. 1 can be obtained.

Table 1-A (around pH = 7.4)

| | $\frac{Na_2HPO_4}{NaH_2PO_4}$ (mEq/l) | NaCl (mEq/l) | I | $\gamma_i$ | pH | E (mV) |
|---|---|---|---|---|---|---|
| 1 | $\frac{27.2}{6.8}$ | 5.8 | 0.05 | 0.812 | 7.473 | 141.05 |
| 2 | $\frac{27.2}{6.8}$ | 11.6 | 0.10 | 0.766 | 7.417 | 149.18 |
| 3 | $\frac{27.2}{6.8}$ | 71.6 | 0.16 | 0.736 | 7.403 | 151.1 |
| 4 | $\frac{27.2}{6.8}$ | 911.6 | 1.0 | 0.689 | 6.842 | 195.59 |

Table 1-B (around pH = 6.8)

| | $\frac{Na_2HPO_4}{NaH_2PO_4}$ (mEq/l) | NaCl (mEq/l) | I | $\gamma_i$ | pH | E (mV) |
|---|---|---|---|---|---|---|
| 5 | $\frac{22.5}{22.5}$ | 5.0 | 0.55 | 0.812 | 6.887 | 177.14 |
| 6 | $\frac{22.5}{22.5}$ | 10 | 0.10 | 0.766 | 6.815 | 185.15 |
| 7 | $\frac{22.5}{22.5}$ | 70 | 0.16 | 0.736 | 6.801 | 187.24 |
| 8 | $\frac{22.5}{22.5}$ | 910 | 1.0 | 0.689 | 6.239 | 254.88 |

Table 1-C

| Calibration Solution | A / B | NaCl |
|---|---|---|
| I | A/B = 4/1<br>0.001 $\sim$ 4 M | 0.001 $\sim$ 4 M |
| II | A/B = 1/1<br>0.001 $\sim$ 4 M | 0.001 $\sim$ 4 M |

( A: $Na_2HPO_4$, B: $NaH_2PO_4$ )

Specifically, when total human blood (with an ionic strength of about 0.15) is dealt with as solution under examination, the electromotive forces of 151.1 and 187.24 mV obtained from pH sensor in calibration solutions I and II in Examples 3 and 7, respectively, are plotted, and a calibration curve with an ionic strength of 0.16, shown by dashed line in Fig. 1 is used.

Suitable ranges of components of calibration solutions I and II are as in Table 1-C as above.

Electromotive force obtained from a pH sensor with saturated sodium chloride calomel electrode (SSEC) as electrode potential pair in case of total human blood was 153.30 mV, and pH at this time measured at a temperature of 37°C using a HL-30 gas monitor manufactured by Radiometer Co., Ltd. was 7.367.

The result copied on the calibration curve of Fig. 1, as shown by the black triangle mark, was identical with the ionic strength of I = 0.16 on the calibration curve.

Where total human blood is the solution under examination, the ionic strength varies with literatures and fluctuates in actual measurements, and this is thought to be due to differences of individuals.

The range of fluctuation may be thought to be 0.08 to 0.18, and a calibration curve may be formed with respect to ionic strength values in this range.

## Second Embodiment

Calibration solutions for simultaneous calibration of ion sensor and gas sensor were prepared as Examples 9 to 18 shown in Tables 2-A and 2-B below. Calibration solution I was used for Examples 9 to 13, and calibration solution II for Examples 14 to 18. $NaHCO_3$ was added to these calibration solutions, and the calibration solution

compositions were calculated on the basis of equation (5).

Mixture gas of $CO_2$ and $O_2$ was dissolved in a ratio of $P_{CO_2}$ = 4.8 kPa (36.1 mmHg) and $P_{O_2}$ = 4.6 kPa (35.2 mmHg) in the solutions of Examples 9 to 13 and in a ratio of $P_{CO_2}$ = 10.5 kPa (79.0 mmHg) and $P_{O_2}$ = 18.9 kPa (142.6 mmHg) in the case of Examples 14 to 18.

The amount of $NaHCO_3$ in calibration solution suited for simultaneous calibration of ion sensor and gas sensor is determined as follows.

As shown in Figs. 2 and 3, by considering equations (4) and (5) the concentration of $HCO_3^-$ in solution was determined from the intersection between curves of equations (4) and (5) by varying $[HCO_3^-]$ alone in the neighborhood of pH = 7.4 (Fig. 2) and in the neighborhood of pH = 6.8 (Fig. 3) while holding constant $[HPO_4^{2-}]/[H_2PO_4^-]$ in equation (4) and $P_{CO_2}$ in equation (5).

Table 2-A (around pH = 7.4, $NaHCO_3$ added)

| | $\frac{Na_2HPO_4}{NaH_2PO_4}$ (mEq/l) | NaCl (mEq/l) | $HCO_3^-$ (mEq/l) | pH | E (mV) | I | $\gamma_i$ |
|---|---|---|---|---|---|---|---|
| 9 | $\frac{27.2}{6.8}$ | 71.6 | 0 | 7.051 | 172.3 | 0.16 | 0.736 |
| 10 | $\frac{27.2}{6.8}$ | 70.6 | 1 | 7.072 | 171.0 | 0.16 | 0.736 |
| 11 | $\frac{27.2}{6.8}$ | 51.6 | 20 | 7.385 | 152.2 | 0.16 | 0.736 |
| 12 | $\frac{27.2}{6.8}$ | 49.2 | 22.4 | 7.414 | 150.5 | 0.16 | 0.736 |
| 13 | $\frac{27.2}{6.8}$ | 21.6 | 50 | 7.621 | 138.1 | 0.16 | 0.736 |

Table 2-B (around pH = 6.8, $NaHCO_3$ added)

| | $\frac{Na_2HPO_4}{NaH_2PO_4}$ (mEq/l) | NaCl (mEq/l) | $HCO_3^-$ (mEq/l) | pH | E (mV) | I | $\gamma_i$ |
|---|---|---|---|---|---|---|---|
| 14 | $\frac{22.5}{22.5}$ | 70 | 0 | 6.598 | 199.4 | 0.16 | 0.736 |
| 15 | $\frac{22.5}{22.5}$ | 69 | 1 | 6.627 | 197.7 | 0.16 | 0.736 |
| 16 | $\frac{22.5}{22.5}$ | 58.2 | 11.8 | 6.860 | 183.7 | 0.16 | 0.736 |
| 17 | $\frac{22.5}{22.5}$ | 50 | 20 | 6.935 | 179.2 | 0.16 | 0.736 |
| 18 | $\frac{22.5}{22.5}$ | 20 | 50 | 7.208 | 168.8 | 0.16 | 0.736 |

Table 2-C

| Calibration Solution | A / B | NaCl | $NaHCO_3$ | $P_{CO_2}$ | $P_{O_2}$ |
|---|---|---|---|---|---|
| III | A/B=4/1 0.005∿4 M | 0.001 ∿4 M | 0.001 ∿0.1 M | 0∼10.6kPa (0∼80 mmHg) | 0∼13.3kPa (0∼100 mmHg) |
| IV | A/B=1/1 0.005∿4 M | 0.001 ∿4 M | 0.001 ∿0.1 M | 5.3∼15.9kPa (40∼120 mmHg) | 6.6∼101kPa (50∼760mmHg) |

( A: $Na_2HPO_4$, B: $NaH_2PO_4$ )

Black circle marks on the curves represent actual measurement values.

Examples 12 was determined to be appropriate as calibration solution III, and Example 16 as calibration solution IV.

Calibration curves for pH sensor, carbon dioxide gas sensor and oxygen gas sensor can be formed by measuring the electromotive forces of the pH sensor and carbon dioxide gas sensor and current in the oxygen gas sensor in both the calibration solutions III and IV, plotting the electromotive forces of the pH sensor and carbon dioxide gas sensor for the pH values and $P_{CO_2}$ values of the solutions III and IV and plotting the current in the oxygen gas sensor for the $P_{O_2}$ values.

Suitable ranges of components of calibration solutions III and IV are as in Table 2-C as above.

By obtaining calibration solution in the above way, producing calibration curves by considering a measurement temperature range of 37°C to 30°C, and memorizing electrode characteristics of sensor at this time, it is possible to provide calibration solution and calibration system suitable for continuous monitoring in biomedical engineering.

Third Embodiment

Figs. 4 to 6 illustrate the status of charges induced on solid membrane electrode surface by ions and/or protein dissolved in solution under examination.

Fig. 4 shows the behavior of sodium ions in the solution under examination containing NaCl with respect to the film surface of an hydrogen ion carrier membrane of a solid membrane electrode immersed in the solution under examination. As shown in (a), sodium ions near the membrane surface are captured on the membrane surface to induce positive charges, while inducing negative charges on the carrier membrane of electrode on the side of conductive base.

The membrane surface potential E and sodium ion concentration are related as shown in (b), and the calibration solution used here is prepared in comformity to the status of charges induced on the membrane surface.

Fig. 5 shows behavior in case where magnesium and calcium ions are dissolved in the solution under examination. As shown in (a), these ions induce negative charges on the hydrogen carrier membrane surface, and the calcium ion concentration, for instance, and membrane surface potential E are related substantially linearly as shown in (b). The same applies in the case of magnesium ions.

The calibration solution in this case, therefore, is prepared such as to conform to the status of charges induced in the membrane surface from the consideration of the relation shown in the graph.

Fig. 6 concerns a case, in which the solution under examination is a protein solution containing dissolved bovine serum albumin (BSA) as protein.

It will be seen that a calibration solution concerning a protein solution as above as the solution under consideration is prepared such as to conform to the status of charges from the consideration of the relation shown in (b).

Examples of electrode subject to the influence of adsorbed protein are Ion-selective field-effect transistor (ISFET) electrodes, platinum electrodes, $SnO_2$ electrodes and glassy carbon electrodes. With ISFET electrodes, in which glass electrode and sensor membrane are covered with glass, variation of protein concentration caused no potential changes.

## Claims

1. A calibration solution for a sensor with a solid electrode for measuring ion concentration of a solution under examination, characterized in that said calibration solution comprises a standard buffer solution which is a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 ratio or in a 4 to 1 ratio, and NaCl being added to said buffer solution to provide an ionic strength substantially identical with the ionic strength of said solution under examination.

2. The calibration solution for a sensor according to claim 1, wherein said sensor is an ion sensor.

3. The calibration solution for a sensor according to claim 2, wherein said sensor is a pH sensor.

4. The calibration solution for a sensor according to claim 1, wherein the ionic strength of said calibration solution is in a range of 0.08 to 0.18.

5. A calibration method for calibrating an ion sensor comprising the steps of :
 setting an ionic strength which is substantially identical with the ionic strength of solution under examination by adding NaCl to a Standard buffer solution which is a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 ratio or in a 4 to 1 ratio, and while preparing at least two reference calibration solutions having different pH values ;
 measuring the electromotive force in each of said reference calibration solutions by immersing said sensor in each said solution ; and
 calculating a calibration formula by plotting the electromotive forces with respect to the pH values of said reference calibration solutions.

6. A calibration solution for a sensor for simultaneously calibrating an ion sensor and a gas sensor, these sensors being provided with solid electrodes with respect to the ion concentration and the dissolved gas concentration in the solution under examination, characterized in that said calibration solution comprises a standard buffer solution which is a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 ratio or in a 4 to 1 ratio, and
 NaCl being added to said standard buffer solution to provide an ionic strength substantially identical with the ionic strength of solution under examination, and a predetermined quantity of bicarbonate buffer solution being added to maintain the ion concentration substantially constant.

7. The calibration solution for a sensor according to claim 6, wherein said bicarbonate buffer solution is $NaHCO_3$.

8. The calibration solution according to claim 7, which contains predetermined quantities of carbon and oxygen gases.

9. The calibration solution according to claim 8, wherein said sensor is a carbon dioxide gas sensor.

10. The calibration solution for a sensor according to claim 9, wherein said sensor is an oxygen gas sensor.

11. A calibration method for simultaneously calibrating an ion sensor and a gas sensor comprising the steps of :
 preparing at least two calibration solutions by adding a bicarbonate buffer solution to at least two standard buffer solutions each composed of a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 or 4 to 1 ratio, having different pH values and partial gas pressure values upon addition of NaCl for setting an ionic strength substantially identical with the ionic strength of the solution under examination, for stabilizing the pH value ;
 measuring the electromotive forces of said ion and gas sensors in said reference calibration solutions by immersing said sensors in said solutions ; and
 calculating calibration formulas by plotting the electromotive forces of said ion gas sensors for the pH values and partial gas pressure values of said standard buffer liquids, respectively.

12. The calibration method according to claim 11, wherein said ion and gas sensors are respectively a pH sensor and a carbon dioxide gas sensor.

13. A calibration method for simultaneously calibrating a pH sensor, a carbon dioxide gas sensor and an oxy-

gen gas sensor comprising the steps of :

preparing at least two calibration solutions by adding a bicarbonate buffer solution to at least two standard buffer solutions each composed of a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 or 4 to 1 ratio, having different pH values, partial carbon dioxide gas pressure values and partial oxygen pressure values upon addition of NaCl for setting an ionic strength substantially identical with the ionic strengt of solution under examination, for stabilizing the pH value ;

measuring the electromotive forces of said pH and carbon dioxide gas sensors and also the current in said oxygen sensor in said reference calibration solutions by immersing said sensors in said solutions; and

calculating calibration formulas by plotting the electromotive forces of said pH and carbon gas sensors for the pH values and partial carbon dioxide gas pressure values of said reference calibration solutions and also calculating a calibration formula by plotting the current value of said oxygen sensor for the partial oxygen pressure value.

14. The calibration method according to claim 11, wherein said bicarbonate buffer solution is $NaHCO_3$.

15. A calibration solution for sensor with a solid electrode for measuring ion concentration of a solution under examination, is a standard buffer solution which comprises a mixture solution of $Na_2HPO_4$ and $NaH_2PO_4$ in a 1 to 1 ratio or in a 4 to 1 ratio, and NaCl being added to said buffer solution to let the solid membrane surface of said electrode to be held at an equilibrium potential.

## Patentansprüche

1. Kalibrierlösung für einen Sensor mit einer Festkörperelektrode zum Messen der Ionenkonzentration einer zu untersuchenden Lösung, dadurch gekennzeichnet, daß die Kalibrierlösung eine Standardpufferlösung umfaßt, die eine Mischungslösung aus $Na_2HPO_4$ und $NaH_2PO_4$ in einem 1:1-Verhältnis oder in einem 4:1-Verhältnis ist, und wobei NaCl zu der Pufferlösung hinzugefügt wird, um eine Ionenstärke bereitzustellen, die im wesentlichen identisch mit der Ionenstärke der zu untersuchenden Lösung ist.

2. Kalibrierlösung für einen Sensor nach Anspruch 1, wobei der Sensor ein Ionensensor ist.

3. Kalibrierlösung für einen Sensor nach Anspruch 2, wobei der Sensor ein pH-Sensor ist.

4. Kalibrierlösung für einen Sensor nach Anspruch 1, wobei die Ionenstärke der Kalibrierlösung in einem Bereich von 0,08 bis 0,18 ist.

5. Kalibrierverfahren zum Kalibrieren eines Ionensensors, umfassend die folgenden Schritte:
Einstellen einer Ionenstärke, die im wesentlichen identisch mit der Ionenstärke der zu untersuchenden Lösung ist, indem man NaCl zu einer Standardpufferlösung hinzufügt, die eine Mischungslösung aus $Na_2HPO_4$ und $NaH_2PO_4$ in einem 1:1-Verhältnis oder in einem 4:1-Verhältnis ist, und wobei mindestens zwei Referenz-Kalibrierlösungen mit unterschiedlichen pH-Werten hergestellt werden;
Messen der elektromotorischen Kraft in jeder der Referenz-Kalibrierlösungen durch Eintauchen des Sensors in jede Lösung; und
Berechnen einer Kalibrierformel durch Auftragen der elektromotorischen Kräfte gegen die pH-Werte der Referenz-Kalibrierlösungen.

6. Kalibrierlösung für einen Sensor zum gleichzeitigen Kalibrieren eines Ionensensors und eines Gassensors, wobei diese Sensoren mit Festkörper-Elektroden ausgestattet sind, in Hinblick auf die Ionenkonzentration und die gelöste Gaskonzentration in der zu untersuchenden Lösung, dadurch gekennzeichnet, daß die Kalibrierlösung eine Standardpufferlösung umfaßt, die eine Mischungslösung aus $Na_2HPO_4$ und $NaH_2PO_4$ in einem 1:1-Verhältnis oder in einem 4:1-Verhältnis ist, und daß NaCl zu der Standardpufferlösung hinzugefügt wird, wobei eine Ionenstärke bereitgestellt wird, die im wesentlichen identisch mit der Ionenstärke der zu untersuchenden Lösung ist, und daß eine vorbestimmte Menge Bicarbonatpufferlösung hinzugefügt wird, um die Ionenkonzentration im wesentlichen konstant zu halten.

7. Kalibrierlösung für einen Sensor nach Anspruch 6, wobei die Bicarbonatpufferlösung $NaHCO_3$ ist.

8. Kalibrierlösung nach Anspruch 7, die vorbestimmte Mengen an Kohlenstoff- und Sauerstoffgasen enthält.

9. Kalibrierlösung nach Anspruch 8, wobei der Sensor ein Kohlendioxidgas-Sensor ist.

10. Kalibrierlösung für einen Sensor nach Anspruch 9, wobei der Sensor ein Sauerstoffgas-Sensor ist.

11. Kalibrierverfahren zum gleichzeitigen Kalibrieren eines Ionensensors und eines Gassensors, umfassend die folgenden Schritte:
Herstellen von mindestens zwei Kalibrierlösungen durch Hinzufügen einer Bicarbonatpufferlösung zu mindestens zwei Standardpufferlösungen, die jeweils aus einer Mischungslösung aus $Na_2HPO_4$ und $NaH_2PO_4$ in einem 1:1-Verhältnis oder in einem 4:1-Verhältnis zusammengesetzt sind, mit unterschiedlichen pH-Werten und Gas-Partialdruckwerten bei Zugabe von NaCl zum Einstellen einer Ionenstärke, die im wesentlichen identisch mit der Ionenstärke der zu untersuchenden Lösung ist, um den pH-Wert zu stabilisieren;
Messen der elektromotorischen Kräfte des Ionen- und Gassensors in den Referenz-Kalibrierlösungen, indem man die Sensoren in die Lösungen eintaucht; und
Berechnen von Kalibrierformeln durch Auftragen der elektromotorischen Kräfte der Ionengassensoren jeweils gegen die pH-Werte und Gas-Partialdruckwerte der Standard-Pufferlösungen.

12. Kalibrierverfahren nach Anspruch 11, wobei die Ionen- und Gassensoren jeweils ein pH-Sensor und ein Kohlendioxidgas-Sensor sind.

13. Kalibrierverfahren zum gleichzeitigen Kalibrieren eines pH-Sensors, eines Kohlendioxidgas-Sensors und eines Sauerstoffgas-Sensors, umfassend die folgenden Schritte:
Herstellen von mindestens zwei Kalibrierlösungen durch Hinzufügen einer Bicarbonatpufferlösung zu mindestens zwei Standardpufferlösungen, die jeweils aus einer Mischungslösung aus $Na_2HPO_4$ und $NaH_2PO_4$ in einem 1:1-Verhältnis oder in einem 4:1-Verhältnis zusammengesetzt sind, mit unterschiedlichen pH-Werten, Kohlendioxid-Partialgasdruckwerten und Sauerstoffpartialgasdruckwerten bei Zugabe von NaCl zum Einstellen einer Ionenstärke, die im wesentlichen identisch mit der Ionenstärke der zu untersuchenden Lösung ist, um den pH-Wert zu stabilisieren;
Messen der elektromotorischen Kräfte des pH- und Kohlendioxid-Gassensors und auch des Stroms in dem Sauerstoffsensor in den Referenz-Kalibrierlösungen durch Eintauchen der Sensoren in die Lösungen; und
Berechnen von Kalibrierformeln durch Auftragen der elektromotorischen Kräfte des pH- und Kohlenstoff-Gassensors gegen die pH-Werte und Kohlendioxidgas-Partialdruckwerte der Referenz-Kalibrierlösungen;
und auch Berechnen einer Kalibrierformel durch Auftragen des Stromwerts des Sauerstoffsensors gegen den Sauerstoff-Partialdruckwert.

14. Kalibrierverfahren nach Anspruch 11, wobei die Bicarbonatpufferlösung $NaHCO_3$ ist.

15. Kalibrierlösung für Sensoren mit einer Festkörperelektrode zum Messen von Ionenkonzentrationen einer zu untersuchenden Lösung, wobei die Kalibrierlösung eine Standardpufferlösung ist, die eine Mischungslösung aus $Na_2HPO_4$ und $NaH_2PO_4$ in einem 1:1-Verhältnis oder in einem 4:1-Verhältnis umfaßt, und wobei NaCl zu der Pufferlösung hinzugefügt wird, um zu erreichen, daß die Festkörper-Membranoberfläche der Elektrode auf einem Gleichgewichtspotential gehalten wird.

## Revendications

1. Solution de calibration pour un détecteur muni d'une électrode solide pour mesurer la concentration en ions d'une solution à examiner, caractérisée en ce que ladite solution de calibration comprend une solution tampon standard qui est une solution d'un mélange de $Na_2HPO_4$ et de $NaH_2PO_4$ dans un rapport de 1 à 1 ou dans un rapport de 4 à 1 et NaCl étant ajouté à ladite solution tampon pour fournir une force ionique sensiblement identique à la force ionique de ladite solution à examiner.

2. Solution de calibration pour un détecteur selon la revendication 1, dans laquelle ledit détecteur est un détecteur d'ions.

3. Solution de calibration pour un détecteur selon la revendication 2, dans laquelle ledit détecteur est un détecteur de pH.

4. Solution de calibration pour un détecteur selon la revendication 1, dans laquelle la force ionique de ladite solution de calibration se trouve dans l'intervalle de 0,08 à 0,18.

5. Procédé de calibration pour calibrer un détecteur d'ions comprenant les étapes consistant:
à ajuster une force ionique qui est sensiblement identique à la force ionique de la solution à examiner en ajoutant NaCl à une solution tampon standard qui est une solution d'un mélange de $Na_2HPO_4$ et de $NaH_2PO_4$ dans un rapport de 1 à 1 ou dans un rapport de 4 à 1 et, tout en préparant au moins deux solutions de calibration de référence ayant des valeurs de pH différentes;
à mesurer la force électromotrice dans chacune desdites solutions de calibration de référence en immergeant ledit détecteur dans chacune desdites solutions; et
à calculer une formule de calibration en traçant les forces électromotrices en fonction des valeurs de pH desdites solutions de calibration de référence.

6. Solution de calibration pour un détecteur pour calibrer simultanément un détecteur d'ions et un détecteur de gaz, ces détecteurs étant munis d'électrodes solides en ce qui concerne la concentration en ions et la concentration en gaz dissous dans la solution à examiner, caractérisée en ce que ladite solution de calibration comprend une solution tampon standard qui est une solution d'un mélange de $Na_2HPO_4$ et de $NaH_2PO_4$ dans un rapport de 1 à 1 ou dans un rapport de 4 à 1 et NaCl étant ajouté à ladite solution tampon standard pour fournir une force ionique sensiblement identique à la force ionique de la solution à examiner, et une quantité prédéterminée de solution tampon de bicarbonate étant ajoutée pour maintenir la concentration en ions sensiblement constante.

7. Solution de calibration pour un détecteur selon la revendication 6, dans laquelle ladite solution tampon de bicarbonate est $NaHCO_3$.

8. Solution de calibration selon la revendication 7, qui contient des quantités prédéterminées de gaz de carbone et d'oxygène.

9. Solution de calibration selon la revendication 8, dans laquelle ledit détecteur est un détecteur de gaz de dioxyde de carbone.

10. Solution de calibration pour un détecteur selon la revendication 9, dans laquelle ledit détecteur est un détecteur de gaz d'oxygène.

11. Procédé de calibration pour calibrer simultanément un détecteur d'ions et un détecteur de gaz comprenant les étapes consistant:
à préparer au moins deux solutions de calibration en ajoutant une solution tampon de bicarbonate à au moins deux solutions tampons standards chacune composée d'une solution de mélange de $Na_2HPO_4$ et de $NaH_2PO_4$ dans un rapport de 1 à 1 ou de 4 à 1, ayant des valeurs de pH et des valeurs de pression partielle de gaz différentes par addition de NaCl pour ajuster une force ionique sensiblement identique à la force ionique de la solution à examiner, pour stabiliser la valeur du pH;
à mesurer les forces électromotrices desdits détecteurs d'ions et de gaz dans lesdites solutions de calibration de référence en immergeant lesdits détecteurs dans lesdites solutions; et
à calculer des formules de calibration en traçant les forces électromotrices desdits détecteurs de gaz et d'ions respectivement en fonction des valeurs de gaz et des valeurs de pression partielle de gaz desdits liquides tampons standards.

12. Procédé de calibration selon la revendication 11, dans lequel lesdits détecteurs d'ions et de gaz sont respectivement un détecteur de pH et un détecteur de gaz de dioxyde de carbone.

13. Procédé de calibration pour calibrer simultanément un détecteur de pH, un détecteur de gaz de dioxyde de carbone et un détecteur de gaz d'oxygène comprenant les étapes consistant:
à préparer au moins deux solutions de calibration en ajoutant une solution tampon de bicarbonate à au moins deux solutions tampons standards chacune composée d'une solution de mélange de $Na_2HPO_4$ et de $NaH_2PO_4$ dans un rapport de 1 à 1 ou de 4 à 1, ayant des valeurs de pH, des valeurs de pression partielle de gaz de dioxyde de carbone et des valeurs de pression partielle d'oxygène différentes par addition de NaCl pour ajuster une force ionique sensiblement identique à la force ionique de la solution à examiner, pour stabiliser la valeur du pH;
à mesurer les forces électromotrices desdits détecteurs de pH et de gaz de dioxyde de carbone

et aussi le courant dans ledit détecteur d'oxygène dans lesdites solutions de calibration de référence en immergeant lesdits détecteurs dans lesdites solutions; et

à calculer des formules de calibration en traçant les forces électromotrices desdits détecteurs de pH et de gaz de dioxide de carbone en fonction des valeurs de pH et des valeurs de pression partielle de gaz de dioxyde de carbone desdites sblutions de calibration de référence et à calculer aussi une formule de calibration en traçant la valeur du courant dudit détecteur d'oxygène en fonction de la valeur de pression partielle d'oxygène.

**14.** Procédé de calibration selon la revendication 11, dans lequel ladite solution tampon de bicarbonate est $NaHCO_3$.

**15.** Solution de calibration pour un détecteur muni d'une électrode solide pour mesurer une concentration en ions d'une solution à examiner qui est une solution tampon standard qui comprend une solution de mélange de $Na_2HPO_4$ et de $NaH_2PO_4$ dans un rapport de 1 à 1 ou dans un rapport de 4 à 1 et NaCl étant ajouté à ladite solution tampon pour permettre à la surface de membrane solide de ladite électrode d'être maintenue à un potentiel d'équilibre.

FIG.1

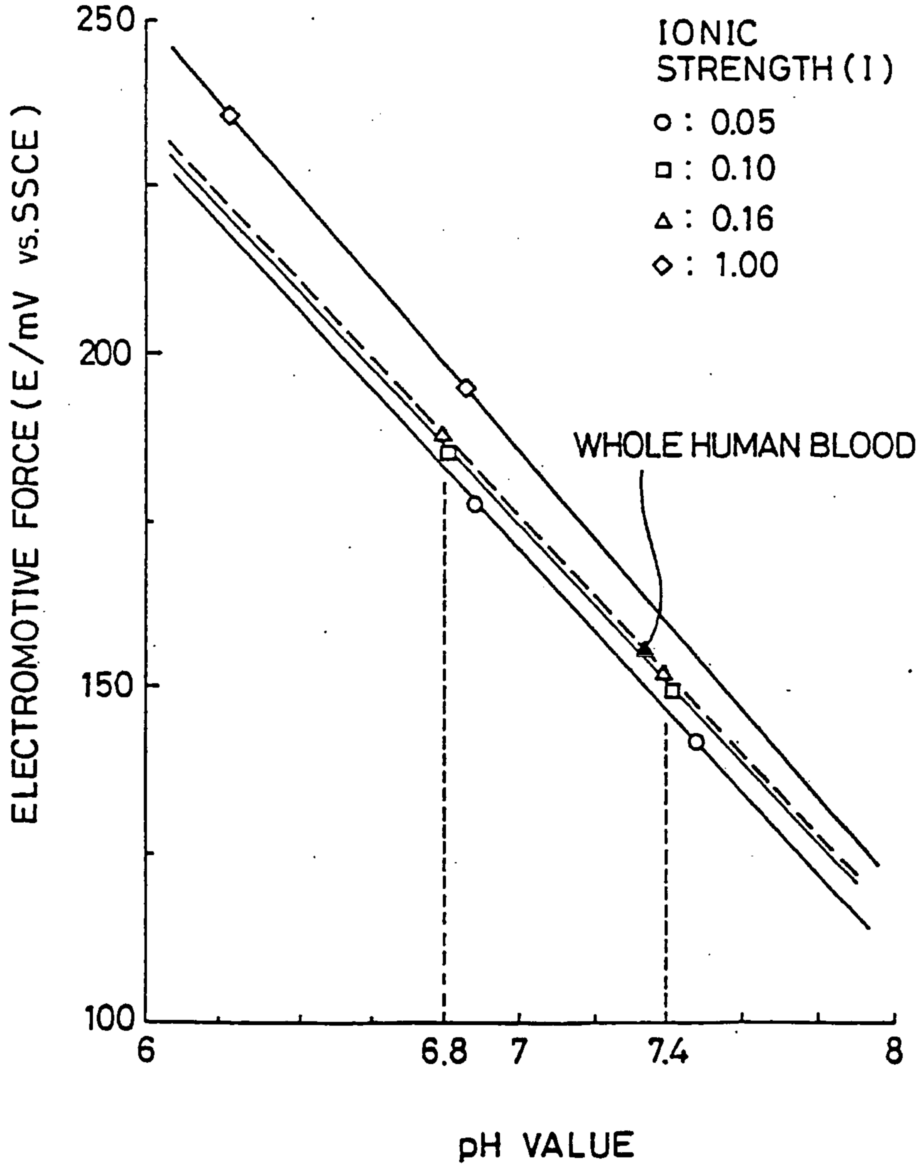

250
200
150
100
ELECTROMOTIVE FORCE (E/mV vs.SSCE)
IONIC STRENGTH (I)
○ : 0.05
□ : 0.10
△ : 0.16
◇ : 1.00
WHOLE HUMAN BLOOD
6
6.8
7
7.4
8
pH VALUE

FIG.2

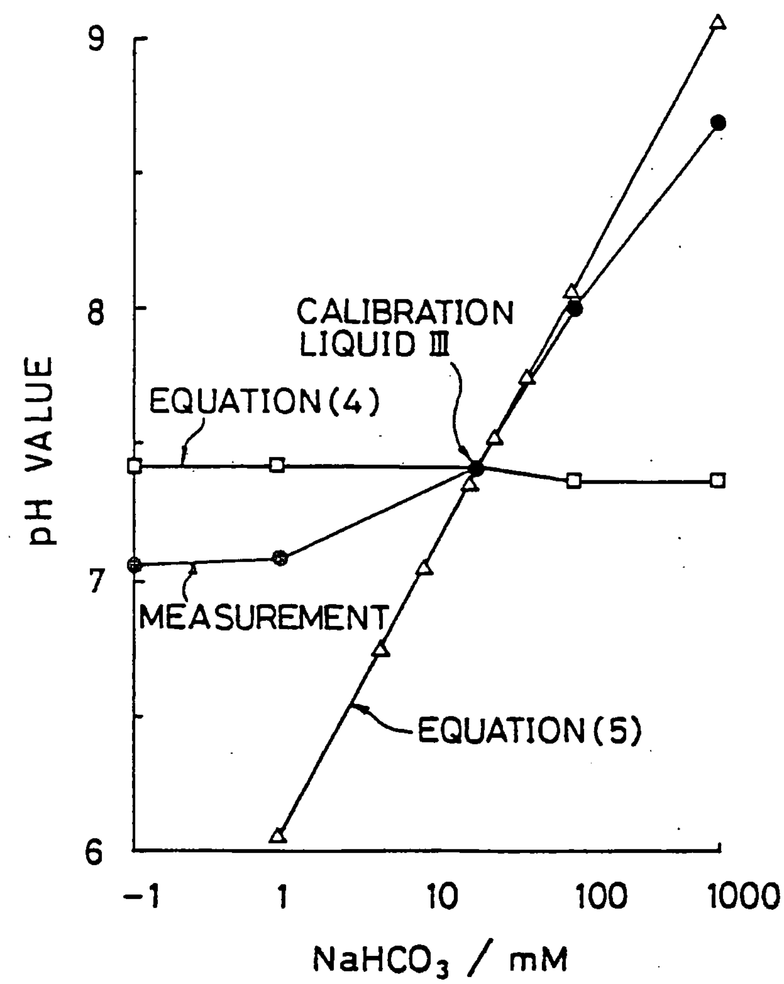

9
8
7
6
pH VALUE
CALIBRATION LIQUID III
EQUATION (4)
MEASUREMENT
EQUATION (5)
−1
1
10
100
1000
$NaHCO_3$ / mM

FIG.3

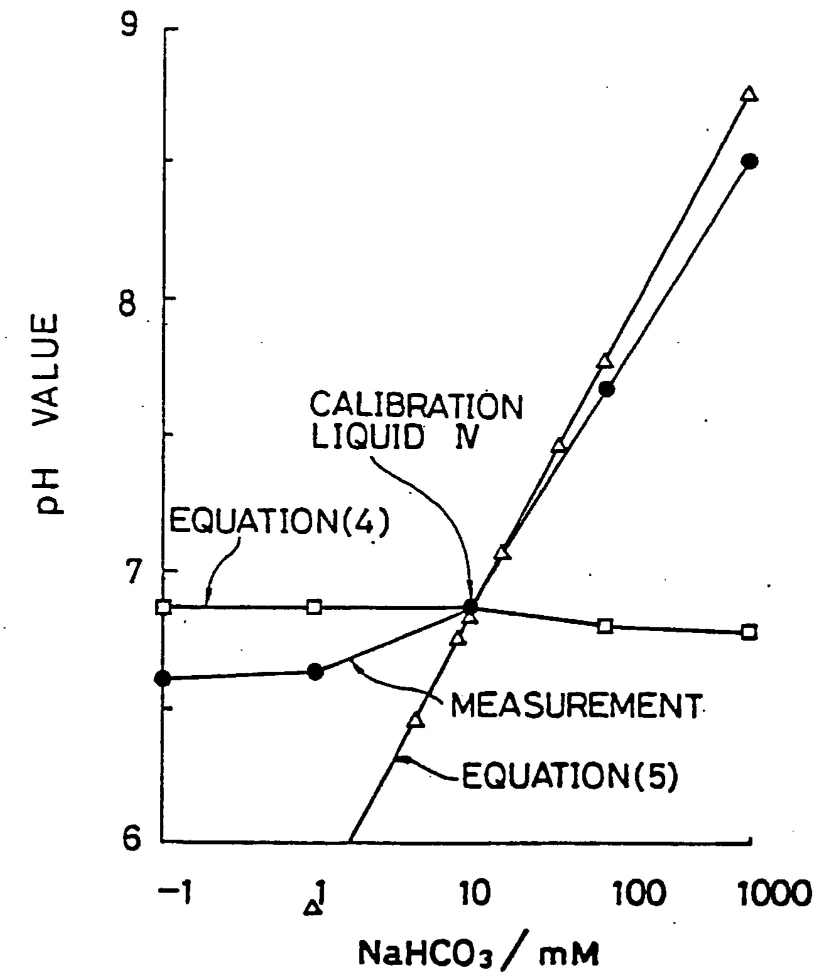

9
8
7
6
pH VALUE
CALIBRATION LIQUID Ⅳ
EQUATION(4)
MEASUREMENT
EQUATION(5)
−1
1
10
100
1000
NaHCO3 / mM

FIG.4(a)

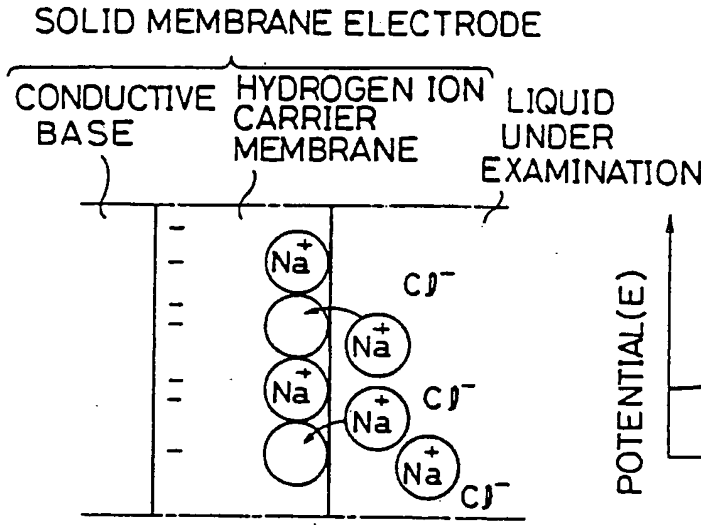



FIG.4(b)

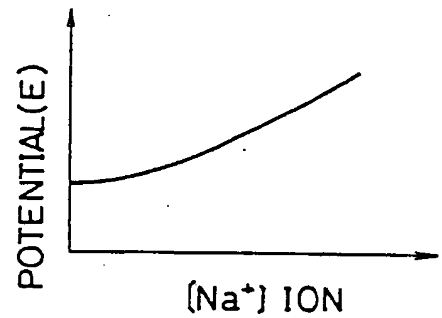



FIG.5(a)

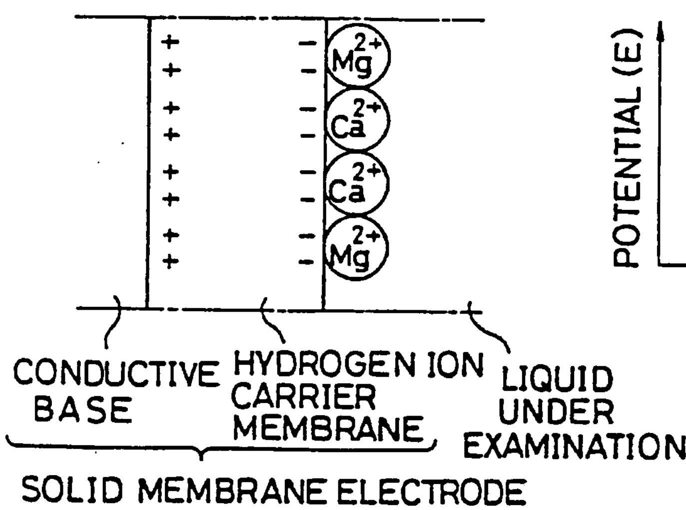



FIG.5(b)

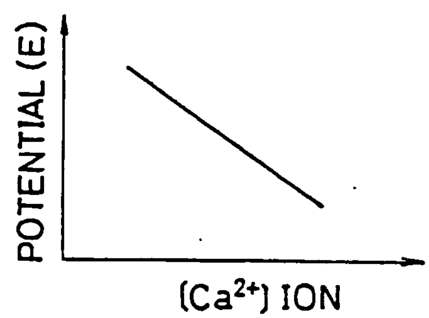


FIG.6(a)

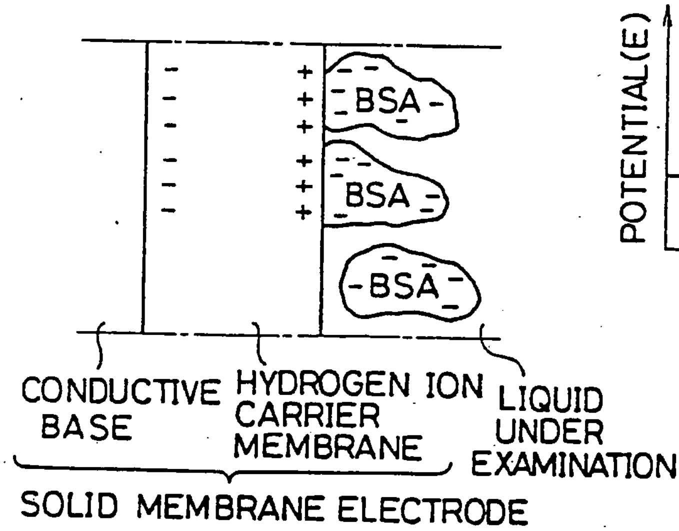
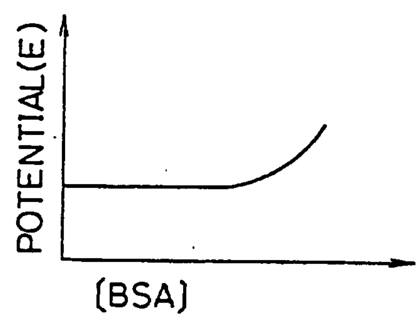

BSA
BSA
BSA
CONDUCTIVE BASE
HYDROGEN ION CARRIER MEMBRANE
LIQUID UNDER EXAMINATION
SOLID MEMBRANE ELECTRODE

FIG.6(b)


POTENTIAL(E)
(BSA)